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Disease-a-Month

The Hemoglobinopathies

WALLACE N. JENSEN

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The Hemoglobinopathies

WALLACE N. JENSEN

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Wallace N. Jensen

is Associate Professor of Medicine and head of the division of hematology, University of Pittsburgh Medical School. He was associated with Drs. Maxwell M. Wintrobe and George E. Cartwright as a Resident and Fellow in Hematology for a period of 5 years and was for 2 years a member of the Faculty of Medicine at Duke University. Dr. Jensen's interests are primarily in blood disorders and medical genetics.

INFORMATION that has accrued during the past decade has resulted in the description of a group of disorders which are currently termed the "hemoglobinopathies."* Included in this group are persons whose erythrocytes contain hemoglobin which differs from the normal either in the proportions of the various normal hemoglobins or in the presence of qualitatively different types of hemoglobin. The hemoglobinopathies are hereditary, and they may or may not be associated with demonstrable physiologic impairment (e.g., hemolytic anemia) in the affected person and may or may not be associated with discernible erythrocytic morphologic alterations. The earliest described and most commonly occurring hemoglobinopathies are sickle cell anemia, thalassemia and their variants. Despite the excellent clinical descriptions and the detailed physiologic and morphologic observations of these disorders, a more complete appreciation of the large variety of hemoglobinopathies has been afforded only by correlated studies of patients, families and large populations and by a detailed chemical study of the hemoglobin molecule. These studies have,

*The terms "abnormal hemoglobin" and "hemoglobinopathy" imply the presence of disease. From this standpoint, they are not ideal, since most of the persons with inherited hemoglobins that are dissimilar to those of the major portion of the population do not exhibit physiologic disadvantage or clinical disease. These terms afford needed facility in communication and are firmly established in the literature. Throughout this paper, the terms do not necessarily denote the presence of disease.

at the same time, provided appreciable information and elicited new, provocative theses concerning the relations between the genetic constitution of the individual, the synthesis of the various proteins, the functions of these proteins and the possible consequent occurrence of clinically demonstrable disease.

Two important studies provided the basis for the extensive chemical and genetic investigations of the past decade. One was the study of Neel (39), which firmly established the relation between the genetic constitution of persons with sickle cell hemoglobin (i.e., heterozygosity or homozygosity) and the occurrence of the clinically quite benign sickle cell trait or the seriously debilitating disease, sickle cell anemia. The second important study was that made by Pauling, Itano, Singer and Wells (41), who described an electrophoretic difference between the hemoglobins of patients with sickle cell anemia and those of normal persons. These investigators demonstrated the presence of both normal and sickle cell hemoglobins in the erythrocytes of patients with sickle cell trait and the presence of only sickle cell hemoglobin in the erythrocytes of patients with sickle cell anemia. Thus, a direct association was made between the occurrence of an abnormal or mutant gene and the presence of an abnormal protein.

While knowledge of the detailed structure of the normal and abnormal hemoglobin molecule has been made possible only by the combined use of a variety of methods for protein characterization, electrophoresis has been the most commonly used procedure for the identification of the various hemoglobins. The direction (toward the anode or cathode) and the rate of migration of the hemoglobin or of the other proteins are largely dependent on the net electrical charge on the protein. The net electrical charge is, in turn, dependent on the difference in pH between the isoelectric point of the hemoglobin and the surrounding or supporting solvent or medium. When the pH of the ambient medium is alkaline with respect to the isoelectric point of the hemoglobin, the hemoglobin exhibits a net negative charge and migrates toward the anode. Conversely, when the pH of the supporting medium is acid with respect to the isoelectric point of the hemoglobin, the hemoglobin exhibits a net positive charge and migrates toward the cathode.

The original description of the different electrophoretic characteristics of sickle cell and normal hemoglobin and the use in many laboratories of filter-paper electrophoresis for examination of red cell hemolysates resulted in the finding of many other types of hemoglobin. When it became apparent that multiple abnormal hemoglobins would be found in various human populations, a system of nomenclature (7) was adopted in which newly described hemoglobins were assigned alphabetical names. Thus, nor-

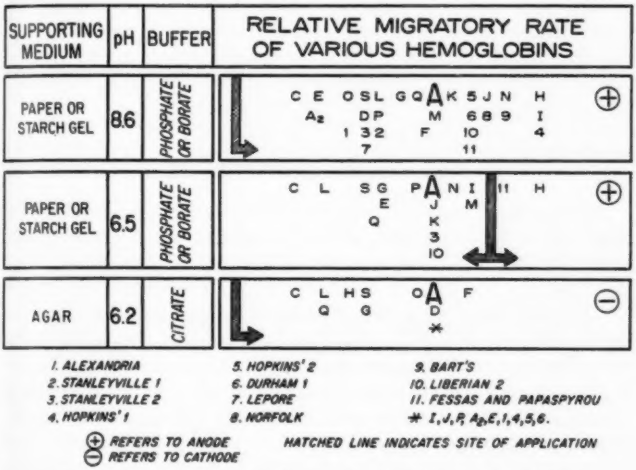


FIG. 1.—Electrophoretic characteristics of the hemoglobins.

mal hemoglobin was designated hemoglobin A, sickle hemoglobin as hemoglobin S, fetal hemoglobin as hemoglobin F, and other hemoglobins as C, D, E, etc. These hemoglobin types may be identified not only by paper electrophoresis but also by electrophoresis on particulate starch, starch gel and acrylamide gel at various pH values. Chromatography, rates of alkaline denaturation, solubility characteristics, analysis of polypeptide and amino acid composition, and dissociation-reassociation of the subunits of the hemoglobin molecule are the other major methods which

have been utilized for the identification of different hemoglobins. To date, approximately 17 different hemoglobins have been described (see Fig. 1, which also shows their electrophoretic characteristics in alkaline [pH 8.6] and acid [pH 6.2] media). Where confusion or indecision has occurred concerning the alphabetical designation which might be appropriate, the hemoglobins have been temporarily identified by appropriate local designations, such as Hopkins-2, Bart's, Alexandria and Liberian-2. These expediencies have become necessary to avoid total confusion in nomenclature, and locally designated hemoglobins are assigned alphabetical names when they are established as being different from those previously identified. The chemical differences, genetic implications and known pathologic concomitants of these abnormal hemoglobins are discussed later in this monograph.

THE HEMOGLOBIN MOLECULE

The methods used for identification of various hemoglobins range from the examination of a peripheral blood smear to the quite complex technic of amino acid analysis of the peptide fragments of the hemoglobin molecule. Paper electrophoresis at pH 8.6 in conjunction with hemoglobin solubility, resistance to alkaline denaturation and a sickle cell preparation sufficed for the differentiation of hemoglobins A, S, C, D, E and G, although these were not ideal methods. Hemoglobins H and I had identical mobilities on paper electrophoresis at pH 8.6 but were readily distinguished by this same procedure at pH 6.5. This emphasized the need to identify and characterize hemoglobins by methods other than those previously utilized. The use of column and paper chromatographic technics in addition to electrophoresis provided the means of identification of certain abnormal hemoglobins. Methods such as agar gel electrophoresis actually combine the technics of electrophoresis and chromatography. The "fingerprinting" method, which will be discussed in the next section, has been an extremely valuable technic, as is demonstrated by the recent analysis of hemoglobin D (19). This hemoglobin was seemingly a single hemogeneous protein which had been described in American Negroes, Chinese, Algerians and Indian Sikhs. Polypeptide-chain and fingerprint analysis of these hemoglobins has shown

that there are at least three different types of hemoglobin D, which have been designated, respectively, as the alpha, beta and gamma types. The use of these methods for the identification of various hemoglobin abnormalities was more or less "forced on" many investigators in this field to allow identification of new hemoglobins; but of equal or greater importance, they have provided rather detailed information concerning the structure of the hemoglobin molecule. The details of the methods used for the identification of hemoglobins are the subject of an excellent recent publication by Jonxis and Huisman (29).

Chemical Structure of Normal Hemoglobins

The chemical structure of the normal human hemoglobins has been described in detail by Schroeder (47), who also gave appro-

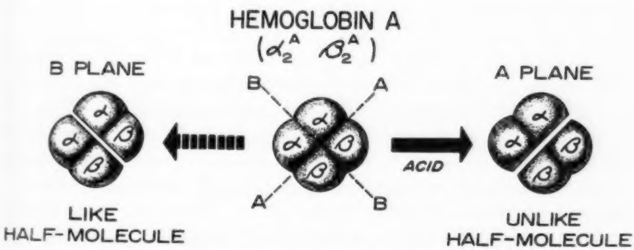


Fig. 2.—Diagrammatic representation of the dissociation of the hemoglobin molecule into subunits.

priate references to the original works. Normal hemoglobin has a molecular weight of approximately 66,700. The intact molecule may be reversibly dissociated into two dissimilar half-molecules, each of which is composed of two similar polypeptide chains (Fig. 2). Each of the four polypeptide chains is associated with a heme group, and the four polypeptide-heme groups are arranged in an ellipsoid mass with a dyad axis of symmetry with dimensions of $55 \times 55 \times 70$ angstrom units. The polypeptide chains of the normal type A hemoglobin molecule are designated as alpha and beta chains, and each molecule contains two alpha chains and

two beta chains. Thus, the normal hemoglobin molecule may be symbolically designated as $\alpha_2{}^A\ \beta_2{}^A$, where the Greek letter refers to the type of polypeptide chain, the subscript to the number of such chains per hemoglobin molecule and the superscript to the normal or abnormal polypeptide chain of the corresponding normal or abnormal hemoglobin.

Erythrocytes of the normal adult contain more than one type of hemoglobin. Approximately 90% is hemoglobin A ($\alpha_2{}^A\ \beta_2{}^A$), and the remaining 10% is composed of minor fractions of types termed fetal hemoglobin, hemoglobin A_2 and hemoglobin A_3. Fetal hemoglobin is present in concentrations of not more than 2%, hemoglobin A_2 in amounts of approximately 2.5% and hemoglobin A_3 in variable quantities. Fetal hemoglobin differs markedly from hemoglobin A chemically and immunologically. It is composed of (*a*) two alpha polypeptide chains apparently identical to the alpha chains of hemoglobin A and (*b*) two gamma polypeptide chains unique to this hemoglobin, designated as $\alpha_2{}^F\ \gamma_2{}^F$. Hemoglobin A_2 may be identified by electrophoresis in an appropriate medium and is composed of two alpha polypeptide chains identical to those of hemoglobins A and F and of two delta polypeptide chains which provide a constitution which differs from the two previously mentioned hemoglobins. Hemoglobin A_2 is designated at $\alpha_2{}^{A_2}\ \delta_2{}^{A_2}$. Hemoglobin A_3 has not been suitably characterized and is thought to be a partially denatured or senescent form of hemoglobin.

The polypeptide chains which form the globin moiety of normal hemoglobins and which have been designated as the α, β, γ, and δ chains have been further chemically degraded and their subunits identified. This has been accomplished in the first stage by an enzymatic digestion of heat-denatured hemoglobin. The enzyme trypsin splits the polypeptide chains at points where arginine or lysine are interspersed and results in the formation of approximately 24–26 smaller peptides. The resultant peptide fragments have been separated and identified by a combination of electrophoresis and chromatography (Fig. 3). A single large piece of filter paper is utilized for electrophoresis of the mixture of peptide fragments in one direction, followed by chromatography in the opposite direction. The peptides are thus separated and arranged in a "map," in which each peptide appears sepa-

rate and in a characteristic site with reference to the other peptides. These peptide maps have been termed "fingerprints" and the technic referred to as "fingerprint analysis" of the protein. The maps are reproducible, and the constancy of position of the peptides *on* the paper suggests a constancy of amino acid composition of the individual peptides. The displacement of a peptide from its usual position indicates a difference in the amino acid composition of that peptide. The individual peptides may then

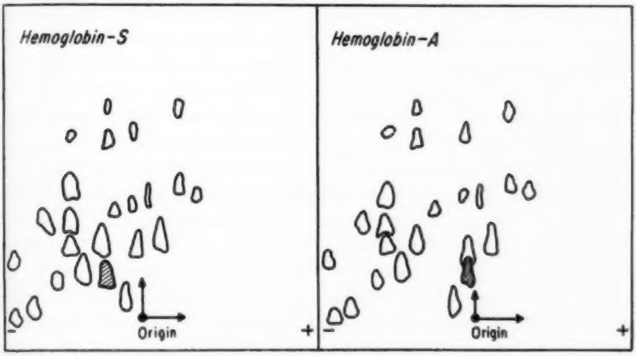


Fig. 3.—Fingerprint patterns of the peptides of hemoglobins A and S. (Courtesy of Dr. Robert L. Hill, Department of Biological Chemistry, University of Utah College of Medicine.)

be further analyzed by subjecting them to sequential amino acid analysis. In this manner, differences in amino acid composition of the various peptides and, inferentially, of the protein molecule have been defined.

Chemical Structure of Abnormal Hemoglobins

The heme radicles of the abnormal hemoglobins have been shown, with the exception of hemoglobin M, to be unaltered from the normal. The variation in relative electrophoretic mobility of sickle cell hemoglobin from the normal hemoglobin has been attributed to differences in the globin moiety of these two

proteins. Repeated attempts to delineate differences in the amino acid composition of sickle cell and of normal hemoglobin were without success prior to the dramatically informative experiments of Ingram and his co-workers (18). These investigators developed and utilized the combination of electrophoresis and chromatography previously described to obtain "fingerprints" of the peptide fragments of the enzymatic digests of the two hemoglobins and were able to demonstrate a difference in their peptides (Fig. 3). Of the 26 peptides identified in normal and sickle cell hemoglobin, 25 were identical. Peptide no. 4 of sickle cell hemoglobin was displaced from the usual position, indicating that the amino acid composition of this peptide differed from that of peptide no. 4 of normal hemoglobin. Sequential amino acid analysis of the no. 4 peptides of normal and sickle cell hemoglobin showed that each had a total of eight amino acids which were identical except for the sixth amino acid. The sixth amino acid of normal type A hemoglobin is glutamic acid, a negatively charged group; whereas in sickle cell hemoglobin the sixth amino acid is valine, which is electrically neutral (20). Thus, the substitution of a valine group for glutamic acid, a change which involves only one of the 300 amino acids in each half-molecule of hemoglobin, provides the basis for the difference between normal and abnormal protein.

Although hemoglobin A ($\alpha_2^A \beta_2^A$) is composed of four polypeptides which form two similar half-molecules, the action of dilute acid on the carbonmonoxy derivative of this same hemoglobin causes the molecule to dissociate into two unlike half-molecules (22) (Fig. 2). The reversible dissociation of hemoglobin into dissimilar half-molecules has been used by Itano and others to characterize the abnormal polypeptide or polypeptides of the hemoglobin molecule.

By dissociation and reassociation of a mixture of molecular species of hemoglobin (i.e., hemoglobins composed of different types of polypeptide chains), it has been possible to identify some of the normal and abnormal peptides as constituents of either the alpha or beta polypeptide chains. In hemoglobin S, the amino acid alteration occurs in the no. 4 peptide, which has been identified as a component of the beta polypeptide chains. In this same hemoglobin the alpha polypeptide chains are similar in

composition to those of normal type A hemoglobin. These interesting methods for dissection and analysis of the normal and the abnormal hemoglobin molecules have indicated that hemoglobin types C, G, E, D_B, J and L, in addition to hemoglobin S, contain amino acid alterations which are located in peptides of the beta polypeptide chains (19, 20, 46). Just as alterations of the beta polypeptide chains are present, it may be expected that some abnormalities reside in the alpha polypeptide chains. Current evidence suggests or has shown that the various abnormal peptides of hemoglobins D_α, I, P, Q and Hopkins-2 are located in the alpha polypeptide chains of these proteins (20, 24).

The occurrence of dual abnormalities of alpha and beta polypeptide chains of a single hemoglobin molecule has also been observed. Thus, in the patient reported by Smith and Torbert (50), whose hemoglobin analysis showed the presence of hemoglobin S ($\alpha_2^A\ \beta_2^S$) and hemoglobin Hopkins-2 ($\alpha_2^{Hop\text{-}2}\ \beta_2^A$), there is evidence that a third molecular species of hemoglobin (composed of the abnormal alpha polypeptide chain of hemoglobin Hopkins-2 and of the abnormal beta polypeptide of sickle hemoglobin) was present. The third molecular type of hemoglobin ($\alpha_2^{Hop\text{-}2}\ \beta_2^S$) had an electrophoretic mobility similar to normal hemoglobin because the differences in the electrical charge of the two abnormal polypeptide chains were of opposite sign and approximately equal magnitude (23).

The combination of four similar polypeptide chains (tetramers) to form hemoglobin molecules occurs only under most unusual circumstances. The reasons for the failure of similar polypeptides, such as α_4 or β_4, to form a hemoglobin molecule are unknown. The only examples of the occurrence of such tetramers are those of hemoglobin H, which is composed of four beta polypeptide chains (β_4^H) and of Bart's hemoglobin, which consists of four gamma polypeptide chains ($\gamma_4^{Bart's}$) (27, 17).

THE INHERITANCE OF HEMOGLOBINS

The early recognition of the occurrence of sickle cell anemia, or of sickle cell trait, in several members and in multiple generations of a single family, plus the nearly total exclusion of this disorder among non-Negro persons, established the familial na-

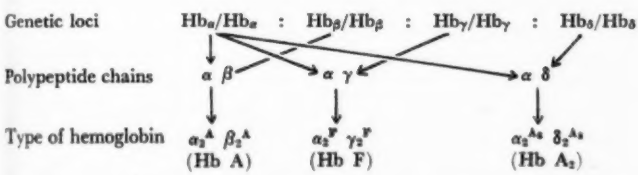
ture of the disease. However, the varied and multiple clinical manifestations of the disease made difficult the formulation of any patterns of inheritance to which there were not almost immediate and multiple objections. The classic genetic studies conducted by Neel (39) and the chemical differences between sickle cell and normal hemoglobin which were described by Pauling, Itano, Singer and Wells (41) provided the basis and the proof for the proposal that sickle cell trait and sickle cell anemia were related, one to the other, as the heterozygous and homozygous states. Integral to this concept was the idea that the type of hemoglobin formed was directed by two genetic determinants (one pair of genes) and that the gene for hemoglobin A and the gene for hemoglobin S were alleles.* Thus, the person with sickle cell trait was heterozygous and formed hemoglobins A and S; the person with sickle cell anemia was homozygous for (and formed only) hemoglobin S, whereas the normal person was homozygous for (and formed only) hemoglobin A.

The inheritance of hemoglobins was, at one time, thought to be directed by a single pair of genes, which were designated as Hb A/Hb A; and the product of these genes was termed normal hemoglobin (type A). Most of the observations of patients and families with hemoglobinopathies were compatible with the suggestion that hemoglobins S, C, D, E and G were the products of genes which were alleles of the hemoglobin A locus. Thus, the sickle cell trait genotype and the hemoglobin C trait genotype were, respectively, Hb A/Hb S and Hb A/Hb C; and mixtures of hemoglobins A and S were found in sickle trait, and mixtures of hemoglobin A and C were found in hemoglobin C trait. In the abnormal homozygous state of sickle cell anemia, the genotype would be Hb S/Hb S with the production of only sickle cell hemoglobin, and in homozygous hemoglobin C disease the genotype would be Hb C/Hb C with the production of only hemoglobin C. Double heterozygous hemoglobinopathies, such as sickle cell–hemoglobin C disease and sickle cell–hemoglobin D disease, were also described.

This concept of the mode of inheritance of hemoglobin determinants was found to be totally inadequate to explain several

*Alleles: contrasting genes situated at the same locus on homologous chromosomes and which determine alternative characters in inheritance.

well-known findings. The developments which at the same time necessitated and provided new ideas for a more satisfactory genetic theory were multiple. These include the presence of at least three chemically different hemoglobins (A, A_2 and F) in the hemolysates of normal erythrocytes (10), the descriptions of persons with more than two qualitatively abnormal hemoglobins in their red cells (50) and the genetic implications of the detailed chemical analyses of the hemoglobin molecule. A newer concept has been proposed, wherein genes are responsible for the formation of *polypeptide chains* rather than the complete hemoglobin-protein molecule (46). The existence of four separate genetic loci is postulated; these loci are, respectively, responsible for the production of alpha, beta, gamma and delta polypeptide chains. The normal complement of genetic loci (genotype) may be symbolically represented as Hb_α^A/Hb_α^A; Hb_β^A/Hb_β^A; Hb_γ^F/Hb_γ^F; $Hb_\delta^{A_2}/Hb_\delta^{A_2}$. In this designation of genetic loci, the superscripts A, F and A_2 refer only to the more common types of hemoglobin formed from the polypeptide products of these loci. Diagrammatically, the normal genetic loci may be related to the normal polypeptide chains and normal hemoglobins in the following manner:



In this schema, a normal person synthesizes four types of polypeptide chains, providing a pool of different polypeptides which, if randomly associated, would result in many other types of hemoglobin than those which have been demonstrated. The association of α, β, γ and δ polypeptide chains to form tetramers apparently does not occur in the normal person, and the great propensity is for the association of dimers of α and β chains which, in combination (α_2 β_2), form the major hemoglobin component of normal erythrocytes. Two exceptions to the usual failure of association of four similar polypeptide chains have already been

mentioned. These occur in the abnormal hemoglobin H molecules, which are composed of four beta polypeptide chains (β_4), and in the abnormal Bart's hemoglobin molecules, which are composed of four gamma polypeptide chains (γ_4).

Current evidence indicates that the genetic determinants for hemoglobins C, D_β, G, E and J are alleles of hemoglobin A and that the abnormality is present in the beta polypeptide chain. In contrast, the genetic determinants for hemoglobins D_α, I, P, Q and Hopkins-2 are thought to be alleles and the abnormality residual to the alpha polypeptide chain. The heterozygous individual with sickle cell trait would then have genotype Hb_α^A/Hb_α^A; Hb_β^A/Hb_β^S; Hb_γ^F/Hb_γ^F; $Hb_\delta^{A_2}/Hb_\delta^{A_2}$ and produce hemoglobin A ($\alpha_2^A\ \beta_2^A$), hemoglobin S ($\alpha_2^A\ \beta_2^S$), hemoglobin F ($\alpha_2^A\ \gamma_2^F$) and hemoglobin A_2 ($\alpha_2^{A_2}\ \delta_2^{A_2}$). The patient with a homozygous hemoglobinopathy such as sickle cell anemia would have the genotype Hb_α^A/Hb_α^A; Hb_β^S/Hb_β^S; Hb_γ^F/Hb_γ^F; $Hb_\delta^{A_2}/Hb_\delta^{A_2}$ and would produce hemoglobin S ($\alpha_2^A\ \beta_2^S$), hemoglobin F ($\alpha_2^A\ \gamma_2^F$) and hemoglobin A_2 ($\alpha_2^{A_2}\ \delta_2^{A_2}$).

The foregoing are examples in which the abnormality is confined to the beta polypeptide; but similar abnormalities of, and parallel descriptions for, the alpha chain also exist. The concurrence of abnormalities involving both alpha and beta chains has been reported by Gerald, Pearson and McCurdy (9). The subject of their report had four identifiable major hemoglobin components demonstrable in his red cell hemolysate. These hemoglobins were analyzed by "fingerprint" and by dissociation-reassociation methods and were found to conform to the four theoretically possible hemoglobins which could result from the production of normal and abnormal beta and alpha polypeptide chains. These were $\alpha_2^A\ \beta_2^A$, $\alpha_2^*\ \beta_2^A$, $\alpha_2^A\ \beta_2^*$ and $\alpha_2^*\ \beta_2^*$. The asterisk refers to abnormal chains, the exact character of which was not described.

Thalassemia, including the variants thereof, requires special consideration. Adequate studies of the subunits of the hemoglobin of this disease have not been performed. The disorder is found primarily in persons who live within, or whose ancestors came from, a geographic beltlike region which includes the Mediterranean basin (particularly the northern shore countries), North Africa, a portion of Central Africa and Southeast Asia. Thalas-

semia major (Cooleys' anemia) and thalassemia minor are thought to be related genetically one to the other, as the homozygous to the heterozygous state. In the patient with thalassemia major, there is usually present in the erythrocytes from 20 to 90% fetal hemoglobin, and hemoglobin A_2, expressed as a fraction of the total, is not increased. In the patient with thalassemia minor, the most common hemoglobin abnormality is the presence of more than 2.5% hemoglobin A_2, and fetal hemoglobin is usually not increased. Other thalassemia disorders, termed "thalassemia intermedia," have usually been found to be double heterozygous states, in which thalassemia minor and one of the other hemoglobinopathies are coexistent. Quite commonly, the double heterozygous state of thalassemia with hemoglobins S, C, E or H has occurred. Theories as to the further heterogeneity of thalassemia, the occurrence of various abnormalities of polypeptide chains and the genetically determined aberrations in the rates of formation of the various polypeptide chains have been offered, but they lack the necessary critical observations for complete evaluation (21).

PHYSIOLOGIC AND CLINICAL CONSIDERATIONS

The most commonly occurring hemoglobinopathies are those which have been called "traits." These refer to heterozygous states, as contrasted with homozygous or double heterozygous states, in which the erythrocytes contain a major fraction of normal type A hemoglobin and usually less than half of the particular abnormal hemoglobin. Hemoglobins A_2 and F are present in normal quantity. With the exceptions of the hemoglobin traits C, D, E and G, in which target cells may be present, there are few or no morphologic alterations of the red cells. The more rarely occurring hemoglobinopathy-trait states (I, J, K, L, N, O, P and Q) have not been associated with specific erythrocyte morphologic abnormality. In none of the hemoglobinopathies except those of sickle cell anemia and hemoglobin M disease have alterations in the capacity for oxygen and carbon dioxide associations and dissociation been described. The abnormalities which occur in these two disorders are discussed later.

The Disorders of Sickle Cell Hemoglobin

The percentage of sickle cell hemoglobin in the erythrocytes of various individuals with sickle cell trait varies considerably. The usual range of sickle cell hemoglobin concentration is from approximately 20 to 45%, but never in excess of 50% of the total amount of hemoglobin. A preponderance of evidence indicates that the red cell population of a given person with sickle cell trait is homogeneous, in that all cells have the same proportions of sickle cell and normal hemoglobin. Patients with sickle cell–hemoglobin C disease have approximately equal amounts of sickle cell hemoglobin and hemoglobin C distributed uniformly within the red cell population. Patients with sickle cell–thalassemia disease differ from those previously mentioned in that the abnormal hemoglobin S usually exceeds 50% of the total. Commonly, there is from 60 to 90% sickle cell hemoglobin; and sickle cell–thalassemia may, in this respect, be indistinguishable from sickle cell anemia. Patients with sickle cell anemia usually have more than 90% hemoglobin S; the remaining hemoglobin is composed of hemoglobins F and A_2, and normal hemoglobin A is absent. There is evidence of minor, but definite, variability of the intracorpuscular concentration of hemoglobin S and F from one cell to another in the erythrocyte population of a patient with sickle cell anemia.

The physiologic consequences of different intraerythrocytic concentrations of sickle cell hemoglobin were at least partially appreciated before the studies of the hemoglobin-molecular alterations in this disease. The relation of the sickling phenomenon to the deoxygenation of blood was described by Hahn and Gillespie in 1927. More quantitative aspects of this relation were provided by the studies of Lange, Minnich and Moore (31), of Harris, Brewster, Ham and Castle (14) and of Griggs and Harris (12). These studies clearly define the in vitro relation between the sickling phenomenon and the partial pressure of oxygen in the erythrocytes of sickle cell trait, sickle cell–hemoglobin C disease, sickle cell–thalassemia and sickle cell anemia.

A critical, comprehensive review of the physical and biologic factors of the sickling phenomenon has recently been published by Harris (13). When samples of blood from patients with sickle

cell anemia are allowed to equilibrate at various concentrations of oxygen (from 0 to 100 mm. Hg O_2) and the percentage of sickled cells is determined, the plot of the percentage of sickled cells against the oxygen tension describes a sigmoid curve. Nearly 100% of the cells are sickled from 0 to 20 mm. Hg O_2; the percentage of sickled cells decreases rapidly in the range of oxygen tension from 20 to 45 mm. Hg, and relatively few sickled cells are found in samples of blood equilibrated at 50 to 700 mm. Hg O_2. In sickle cell–hemoglobin C disease and sickle cell–thalassemia, the blood of patients with lower concentrations of sickle cell hemoglobin exhibits fewer sickled cells at similar oxygen tensions. In contrast with these findings, the blood of patients with sickle cell trait exhibits virtually no sickled cells until the oxygen tension is reduced below 15–10 mm. Hg. The sickling observations are nicely paralleled by changes in the mechanical fragility of erythrocytes and the blood viscosity at these same oxygen tensions. Thus, when oxygen tension is reduced and the numbers of sickled cells increase, the viscosity of the red cell suspension is increased and there is an increased proportion of cells with greater mechanical fragility.

Studies of the behavior of solutions of sickle cell hemoglobin gave an understanding of some of the aspects of the sickling phenomenon. The low solubility and the strikingly increased viscosity of solutions of deoxygenated sickle cell hemoglobin, when compared with normal reduced hemoglobin, oxyhemoglobin and oxygenated sickle cell hemoglobin, suggested a change in the physical orientation of the hemoglobin molecule. In concentrations of deoxygenated sickle cell hemoglobin from 10 to 25 Gm./100 ml., these changes were very striking; and at the higher concentrations, a gel-like state occurred and spindle-like bodies similar to crystals were visible in the gel by phase microscopy. The bodies which were formed in more concentrated solutions of deoxygenated sickle cell hemoglobin exhibited birefringency and were called "tactoids." This same birefringency may be observed in deoxygenated intact sickled erythrocytes. The more important factors in the sickling phenomenon are the intracellular concentration of sickle cell hemoglobin, the state of oxygenation or deoxygenation of the hemoglobin and, to a lesser degree, the pH of the suspending medium of the cells.

From these in vitro observations, it was proposed that the major findings of hemolytic anemia and vascular thrombosis in the sickle cell hemoglobin diseases result from the occurrence, in sequence, of deoxygenation of the erythrocyte, production of sickled cells, an increase in blood viscosity and the resultant slowing of capillary blood flow. At the same time, the sickled cells are prone to lysis because of their increased mechanical fragility. The ultimate result of this chain of events is the occurrence of intravascular thrombosis and premature destruction of erythrocytes.

Studies of in vivo sickling have been made on blood obtained directly from peripheral arteries or veins, or from organ puncture and the use of an intravenous catheter to obtain samples of blood from the venous effluent of specific organs (26). The numbers of sickled cells which were found agreed quite well with those which would be expected from the in vitro experiments. Major deviations between the number of sickled cells predicted by in vitro experiments and those observed in vivo occurred only at the extremes of blood oxygen content. Thus, fewer sickled cells were present in blood with very low oxygen content which was taken from the coronary sinus, and more sickled cells were found in arterial blood than was predicted from the in vitro studies. The number of sickled cells in venous blood from hepatic, renal and jugular veins, the inferior vena cava, right atrium, pulmonary artery and right ventricle was quite constant in the same person despite modest variations in oxygen content of the blood obtained from these anatomic sites. Fewer than 30% sickled cells were found in the various blood samples from the 10 patients studied. The discrepancies between the in vitro and in vivo observations indicate the possible existence of other important factors in the sickling process.

The interval of time required for sickling after deoxygenation of hemoglobin, or for reversion to normal shape upon oxygenation of blood, is quite probably an important facet of in vivo sickling. Extensive and definitive studies relevant to this point have not been done, but it has been estimated that the sickling process requires about 2–4 minutes (2). An accelerated rate of blood flow, imposed by the anemic state, and the shift of the oxygen dissociation curve of the hemoglobin of sickle cells toward the right may be important factors in the prevention of in vivo

sickling and consequent vascular thrombosis. The propensity to thrombosis in the medullary parts of the bone and in the spleen, where blood flow may be comparatively slow, supports this concept.

The observations of multiple thrombosis in patients with sickle cell–hemoglobin C disease, in those with sickle cell–thalassemia disease and occasionally in patients with hemoglobin C disease (where intracellular concentrations of sickle cell hemoglobin are less than in sickle cell anemia) do not conform as well as might be expected to the concepts just outlined. Other observations, such as the hyposthenuria (impairment in renal concentrating ability) exhibited by some persons with sickle cell trait, as well as by patients with sickle cell anemia, and the propensity to retinal vascular abnormalities of persons with sickle cell variants, may not be completely explainable on the basis of intravascular sickling alone.

Hemolytic anemia occurs in sickle cell anemia and the double heterozygous sickle cell states, but it is not an expected finding in sickle cell trait. Patients with sickle cell anemia usually, but not always, have a more severe hemolytic anemia than do those with sickle cell variants. The hemolytic anemia is usually moderately severe and is attended by a markedly shortened erythrocyte life span, reticulocytosis, hyperbilirubinemia, increased stool urobilinogen and normoblastic hyperplasia of the bone marrow. It is unlikely that wide fluctuations in the rate of destruction of the cells occur, since repeated red cell survival determinations in each of 5 patients were similar during a 3-year period of observation (25). The continuous administration of high oxygen concentrations to a group of patients with sickle cell anemia during periods ranging from 8 to 20 days resulted in a decrease in arterial and venous sickled cells, but there was no evidence of altered rates of red cell destruction (44). The major effect of exposure to high oxygen tension was one of decreased erythrocytogenesis and subsequent decreased peripheral red blood cell concentration.

Variations in the red cell counts do occur from time to time, occasionally without apparent reason. The more severe episodes of aggravated anemia may occur in association with hypoplastic or aplastic crises. During such periods, the bone marrow produces few red cells and more severe anemia rapidly develops. Occa-

sionally, folic acid deficiency and an aggravated anemia therefrom have been noted in these disorders, but this state differs from the aplastic crisis in that the marrow remains cellular and shows typical megaloblastic changes. The folic acid deficiency has been ascribed to the combination of inadequate supplies of folic acid and an increased utilization of folic acid imposed by the accelerated hemopoiesis (28).

The physiologic derangements discussed thus far are those which are part of the sickling phenomenon, vascular thrombosis and anemia. The cardiovascular and hemodynamic changes which occur are largely those attributable to chronic severe anemia and to multiple thrombotic episodes, particularly of the pulmonary vascular tree. The decrement in oxygen-carrying capacity resulting from anemia is at least partly compensated by an increase in cardiac output and an increased rate of peripheral blood circulation. The flow of blood resulting from the increased cardiac output is proportionately distributed to all areas of the body, with perhaps the exception of the skin and kidneys, where flow may be decreased.

Another compensatory physiologic mechanism which is utilized in the patient with sickle cell anemia is the increased extraction of oxygen from blood by peripheral tissues. Under basal conditions, these mechanisms allow an approximately normal rate of oxygen consumption. The constant utilization of these compensatory mechanisms to provide adequate peripheral oxygenation is not, however, without cost to the patient. Cardiac hypertrophy and dilatation are almost constant findings in patients with sickle cell anemia, and cardiac failure is a not uncommon cause of death.

An additional factor in the genesis of cardiovascular disease is the occurrence of multiple pulmonary thromboses and infarcts. Repeated episodes of pulmonary vascular thromboses, with or without pulmonary parenchymal infarction, may compromise the total pulmonary vascular bed and produce pulmonary arterial hypertension, right ventricular enlargement and eventually the clinical picture of cor pulmonale. It must be pointed out that most adult patients with sickle cell anemia who have cardiomegaly and accentuation of the second pulmonic sound have not had demonstrable pulmonary artery hypertension when they have been subjected to cardiac catheterization and pulmonary artery pres-

sure measurements (25, 34). The reason for the routine finding of a loud second pulmonic sound are still obscure.

Most patients with sickle cell anemia have modest arterial oxygen desaturation (26, 34). In some patients this may be attributed to pulmonary disease, which has produced diffuse parenchymal damage and pulmonary gaseous diffusion defects, or to anatomic or physiologic pulmonary arteriovenous shunts. There is, however, evidence that the arterial desaturation is partly a function of the abnormal sickle-hemoglobin-laden erythrocyte. Under both in vivo and in vitro conditions, the oxygen dissociation curve of intact erythrocytes is shifted toward the right. The displacement toward the right of the oxygen dissociation curve of sickle-hemoglobin-laden erythrocytes describes a need for a higher oxygen tension to achieve a given degree of hemoglobin oxygen saturation of these cells as compared with normal erythrocytes. The shift toward the right of the oxygen dissociation curve of intact cells containing sickle cell hemoglobin is of sufficient degree to account for the arterial blood oxygen desaturation (45). The lack of a similar alteration of oxygen dissociation in solutions of sickle cell hemoglobin suggests that other cellular substances are of importance in the process of oxygen association and dissociation (4).

The constant hemolysis which takes place in sickle cell anemia, and to a lesser degree in the sickle cell variants, is accompanied by increased hemopoiesis and an increase in the mass and perhaps in the unit activity of the erythropoietic tissues. Frequently there is associated leukocytosis and thrombocytosis. The leukocytosis is often modest in degree, but it is important to recognize the condition as part of the sickle cell anemia, for otherwise it may be assumed to be evidence of complicating disease.

The possible role of the thrombocytosis in the genesis of the thrombosis has not been adequately studied. Blood coagulation studies in sickle cell anemia have not revealed notable abnormalities. Erythrocytes and platelets contain qualitatively similar phospholipids, among which are phosphatidyl ethanolamine and phosphatidyl serine. These compounds have been shown, by various investigators, to possess thromboplastic function. It has been suggested, therefore, that the intravascular destruction of erythrocytes in hemolytic anemia may provide quantities of thrombo-

plastic substances which are capable of either the initiation or the potentiation of intravascular thrombosis. Comparative studies of total lipid, cholesterol, phospholipid and the phospholipid fractions in the erythrocytes and plasma of normal persons and of patients with sickle cell anemia show that the total phospholipid and the various phospholipid fractions are similar in a normal young erythrocyte population and in sickle cells (53).

The erythrocytes of a person with sickle cell anemia are a youthful population of cells compared with those of the normal person. They are, therefore, on the average, larger cells; and they are of lower specific gravity and have increased amounts of water, total lipid, phospholipid and cholesterol. The plasma of patients with sickle cell anemia contains decreased amounts of total lipid and cholesterol. These findings are not peculiar to sickle cell anemia alone but are also found in patients with hemolytic anemia due to other causes. Whether or not the intravascular lysis of erythrocytes contributes in any manner to intravascular thrombosis cannot be stated at the present time. During a study of the renal clearance of hemoglobin in a group of 10 patients with sickle cell anemia, the products of the lysis by distilled water of 100–200 ml. of native whole blood were infused intravenously over a period of less than an hour (33). This was sufficient to produce peak plasma hemoglobin concentrations from 100 to 250 mg./100 ml. and did not produce noticeable immediate or delayed untoward effects in the patients studied.

Another probable consequence of the constant hemolytic process is an elevation of plasma benzidine-reactive pigments. The plasma hemoglobin (which may represent some hemoglobin–plasma protein complexes and methemalbumin) level is elevated; it usually ranges from 4 to 30 mg./100 ml. The serum proteins termed "haptoglobins" are usually absent in patients with sickle cell anemia or sickle cell variants and are usually present in sickle cell trait (32). These alpha-2 proteins are of different types, but they have in common the ability to form complexes with extracorpuscular hemoglobin. The most common types of haptoglobins have been termed types 1-1, 2-1 and 2-2. The type of haptoglobin present in a person is genetically determined; types 1-1 and 2-2 are thought to represent the homozygous states for the genes

Hp^1/Hp^1 and Hp^2/Hp^2, whereas type 2-1 represents the heterozygous state and genotypically is represented as Hp^2/Hp^1.

Haptoglobins are present in a majority of normal persons in amounts which are capable of binding approximately 100–130 mg./100 ml. of extracorpuscular hemoglobin. Haptoglobin levels may be decreased; or they may be absent as the result of genetic constitution (the so-called type O-O), severe liver disease, the intravenous infusion of extracorpuscular hemoglobin solutions or in the presence of moderately severe or severe hemolytic anemia of any cause (38). The physiologic function of haptoglobins is yet to be fully described; but, as a result of their capacity to irreversibly bind extracorpuscular hemoglobin and, thus, to form a protein complex of considerably greater molecular weight than hemoglobin, they regulate to a large degree the plasma concentration of hemoglobin at which this protein enters the glomerular filtrate. Only free plasma hemoglobin is excreted into the glomerular filtrate, and hemoglobinuria occurs only when the concentration of extracorpuscular hemoglobin exceeds the hemoglobin-binding capacity of haptoglobin.

Because the haptoglobin levels in sickle cell anemia and sickle cell variants are near or at zero, one might expect hemoglobinuria to be a common finding in these diseases. This is not the case. A recent study of the renal excretion of hemoglobin in sickle cell anemia provides the physiologic basis for the nonoccurrence of hemoglobinuria in most patients with sickle cell anemia, despite the absence of haptoglobins (33). The rate of glomerular filtration of free hemoglobin was found to be the same in patients with sickle cell anemia as in normal persons. Renal tubular reabsorption was more variable than in normal persons and in some patients exceeded normal values. The renal threshold for free hemoglobin was, therefore, slightly higher than normal, with a mean of 52 ± 29 mg./100 ml. The usual total level of benzidine-reactive substance in the plasma (hemoglobin, methemoglobin, methemalbumin) of patients with sickle cell anemia ranges from 4 to 30 mg./100 ml.; the free hemoglobin level is usually insufficient to produce hemoglobinuria. Whether or not the apparently increased capability for renal tubular reabsorption of hemoglobin is an adaptive response to the continued glomerular filtration of increased amounts of hemoglobin is unknown.

Other renal abnormalities are common in sickle cell disease. Almost all patients with sickle cell anemia and sickle cell variants and a proportion of those with sickle cell trait have an inability to elaborate urine of high osmolality (hyposthenuria) in response to water deprivation or pitressin administration. The physiologic basis of this impairment in renal concentration ability is unknown. Because this defect may be partly reversible in young patients with sickle cell anemia when the hematocrit is elevated by transfusion with normal blood, it has been suggested that the hyposthenuria is related to the sickling phenomenon or to effects of sickle hemoglobin on the kidney (30). However, others have suggested that this defect results from a genetic defect in renal function which is independent of the hemoglobin abnormality. The exact mechanism of this interesting disturbance awaits further study. Renal hemorrhage with hematuria occurs in patients with the various sickle diseases, including sickle cell trait (48). Peculiarly, this occurs almost exclusively in the left kidney, which suggests that some gross anatomic arrangement is important in the genesis of renal epistaxis. The bleeding episodes have been thought to be related to sickling with vascular thrombosis in the renal circulation. The occurrence of this phenomenon in the renal circulation in sickle cell trait, which should require virtually complete deoxygenation of the cell, has been attributed to the possibly unusual circulation of that organ, wherein blood of different red cell concentrations perfuse different anatomic portions. If the medullary portion of the kidney is perfused with blood of relatively low red-cell concentration, as has been proposed by Pappenheimer and Kinter (40), then the cortical circulation would receive blood with a higher hematocrit. This increased concentration of erythrocytes could result in a slower local circulation with greater degrees of deoxygenation of the blood and subsequent sickling with thrombosis. This proposal, however, is not based on physiologic fact.

Regardless of the pathogenesis of the sickling phenomenon and the relation of the sickle phenomenon to intravascular thrombosis, the major pathology of the disease is one of either thrombosis of multiple vessels or ischemic infarction of tissues. These pathologic findings have occurred in all parts of the vascular tree and consequently have given rise to varied symptoms and physical findings.

In those regions where blood flow is perhaps slow because of the presence of multiple sinusoidal pools, infarctions seem to be more common. The spleen is almost always a site of infarction, and a high proportion of patients have medullary bone infarcts. Thrombosis of cutaneous vessels are probably important in the production of the leg ulcers, which are common; and thromboses of the corpora cavernosa are frequent causes of priapism. When thromboses occur in the synovial vessels, a picture of arthralgia and/or overt arthritis may result.

Ocular abnormalities are common, occurring in almost all patients with sickle cell anemia and sickle cell variants. They have been found perhaps even more constantly in patients with sickle cell–hemoglobin C disease than in sickle cell anemia. These findings are the subject of a recent monograph by Lieb, Geeraets and Guerry, in which the results of observations on 65 patients are presented (35). The most common abnormalities were found in the ocular blood vessels. These include conjunctival telangiectasia, widened tortuous retinal veins with the formation of horseshoe-shaped venous patterns, neovascularization, microaneurysms, venous thromboses, retinal hemorrhages, sheathing of retinal vessels and vitreous hemorrhages. Retinal degeneration or atrophy, cholesterol deposits and papilledema were also noted in this group of patients. These investigators classified each case according to the severity of the retinopathy and then divided their material into four categories, ranging from lesser to more severe ocular pathology. Significant differences between sickle cell anemia and sickle cell–hemoglobin C disease were not evident. Good correlation was found between the clinical severity of the disease and the severity of the ocular pathologic findings. The vascular changes in the eye were attributed, by these investigators, to the occurrence of either stasis of blood flow or thrombosis, with resultant ischemia or infarction of tissues.

Sickle cell trait occurs in approximately 10% of the American Negro population and varies widely in the Negro population of Africa. The inferences from and the uses to which the population surveys of the incidence of this abnormality have been placed are discussed in another section. The question, however, of whether or not sickle cell trait is benign is still controversial. In the absence of circumstances which result in hypoxia, clinical and pathologic

experience supports the belief that sickle cell trait is benign. Observations to the contrary continue to appear in which morbidity, or even mortality, has been attributed to sickle cell trait. The previously mentioned hyposthenuria and the "idiopathic" gross hematuria which is inconstantly present in sickle cell trait have no satisfactory explanations. Other data in sickle cell trait which might suggest associated morbidity include a reported decrease in the incidence of sickle cell trait among older American Negroes and the occasional report of sudden death due to cerebral thrombosis. Most of the thromboses in persons with sickle cell trait have occurred only when, for various reasons, the person has been in a state of hypoxia. Splenic infarction is now a well-recognized hazard to the person with sickle cell trait as well as to those persons with sickle cell variants. Flight in unpressurized planes at 10,000 feet without provision for increased oxygen tension will apparently induce vascular thrombosis in persons with sickle cell trait. The unique circulation of the spleen, with opportunity for slow circulation through the sinusoids and hemoconcentration within splenic pulp, provides the basis for the particular susceptibility of this organ to thrombosis.

Hemoglobinopathies Due to Hemoglobins C, D and E

Hemoglobin C trait has an incidence of about 2% among American Negroes; but in certain areas of Africa, such as Ghana, the incidence is as high as 20%. The trait is not accompanied by known disease or by physiologic disadvantage; usually it is manifest only by the presence of target cells in the peripheral blood or an appropriate electrophoresis or chromatography of the hemoglobin. Homozygous hemoglobin C disease has been found usually, but not exclusively, among Negroes. The disorder is characterized by a mild chronic hemolytic anemia, splenomegaly, often hepatomegaly and occasional arthralgias without objective evidence of arthritis. Blood examinations show target cells, a mild anemia, a normoblastic hyperplasia of the bone marrow, an increased osmotic resistance of erythrocytes to hypotonic solutions and, inconstantly, intraerythrocytic hemoglobin crystals. The latter are probably not peculiar to hemoglobin-C-containing erythro-

cytes but may also be seen under appropriate conditions in cells containing a variety of other hemoglobins (1).

Hemoglobin D trait has widespread distribution but is of infrequent occurrence. It has been estimated to have an incidence of 0.4% among American Negroes and of approximately 2% among Algerian Moslems and the Sikhs of North Central India. No known hematologic or clinical abnormalities are associated with this hemoglobin. Homozygous hemoglobin D disease is asymptomatic, but a mild microcytic normochromic anemia is present. Of considerable interest is the finding, previously mentioned, that the first three studied cases of hemoglobin D, which were analyzed by "fingerprinting" and molecular dissociation, were found to differ from each other. Although the hemoglobins from these three patients were identical on electrophoretic analyses, they were, indeed, different types of hemoglobin.

Hemoglobin E has been found among Indonesians, Burmese, Etiturks, Bengalis and Malayans in incidences as high as 12%. The trait is benign, and hemoglobin E disease is likewise quite asymptomatic; there is, however, a mild hemolytic anemia, as evidenced by decreased red cell survival and modest bone marrow erythroid hyperplasia. The spleen is rarely enlarged. The coexistence of thalassemia and hemoglobin E in Southeast Asia gives rise in that area to many cases of the more severe clinical disease, hemoglobin E–thalassemia.

Hemoglobins G, I, J, K, L, N, O, P, Q, Hopkins-1, Hopkins-2, Stanleyville-1, Stanleyville-2, Liberian-2, Durham-1, Lepore and Norfolk have each been described in a few cases, most of which have occurred in combination with normal hemoglobin. The paucity of clinical and genetic information concerning these hemoglobinopathies precludes further description. The hemoglobins designated as Bart's and the fast hemoglobin of Fessas and Papaspyrou (termed Alexandria) occur rarely; they have been found in either the umbilical cord or in the blood of infants. No clinical disorders have been associated with these hemoglobins.

Hereditary Hemoglobin M Disease

Hemoglobin M (hereditary methemoglobinemia) refers to an abnormal pigment, first described in 1948 by Hörlein and Weber

(16), which has faulty oxygen association attributable to an inherited defect of the globin portion of the hemoglobin molecule. These investigators clearly differentiated this type of methemoglobin from those in which methemoglobin accumulates as a consequence of decreased or absent erythrocyte diaphorase or methemoglobin reductase. This type of methemoglobin is not associated with hemolytic anemia or with other hematologic abnormalities, but cyanosis is present. Patients with hemoglobins (and clinical pictures) similar to those of Hörlein and Weber have been described by Gerald (8) and by Pisciotta, Ebbe and Hinz (43). Gerald was able to separate the hemoglobin of his patient by electrophoresis on particulate starch at pH 7.0 into two components; one was electrophoretically and spectroscopically normal, and the other had a lesser cathodal mobility and the spectroscopic identity of methemoglobin. Two other variant types of hemoglobin M are known, for which geographic designations of hemoglobin B (Boston) and hemoglobin M (Milwaukee) have been suggested. Possibly, there are even other variants. Only the trait state, or heterozygous state, has been reported; and about 15–30% of the total hemoglobin is type M.

The Thalassemia Disorders

Lack of information concerning the detailed structural and chemical abnormalities of the hemoglobin molecule in the thalassemia syndromes, the variability of the clinical findings and some inconstancy of correlation between the known abnormalities of hemoglobin and the severity of the disease process preclude a totally satisfactory classification of the thalassemia disorders. Whether or not thalassemia is classified as a hemoglobinopathy, the similarities of thalassemia and the disorders previously discussed (and, indeed, the concurrence of thalassemia and hemoglobins S, C, D, E, G, H, etc., within the same populations and in one person) make necessary a brief assessment of thalassemia.

Until relatively recently, the term "thalassemia" or "Mediterranean anemia" was quite appropriate, for the affected persons were of Italian, Greek, Syrian or Armenian ancestry or were native to Italy, Sicily, Greece, Crete, Cypress, Syria or Turkey. Occasional cases of thalassemia in non-Mediterranean persons

were reported before 1950. These early indications of a more widespread occurrence of thalassemia have received solid confirmation through extended population studies. Recent studies have established the existence of thalassemia in high incidence among the populations of not only the northern and eastern coasts of the Mediterranean Sea but of the entire Mediterranean area, southern India, Thailand, Ceylon, Burma, the Philippines, the Belgian Congo and also in American Negroes. Thus, thalassemia occurs predominantly in the inhabitants of a geographic region which forms a wide belt oriented in an east-west direction extending from the Mediterranean bowl on the west through Southeast Asia on the east. This region has been appropriately referred to by Chernoff as the "thalassemia belt" (6).

Thalassemia minor is a relatively common disorder which is not debilitating or associated with discrete symptomatology. In about 50% of the patients, the spleen is palpably enlarged; and, in most of these patients, there are morphologic abnormalities of erythrocytes discernible on routine peripheral blood smear examination. In the absence of anemia, reticulocytosis or leukocyte abnormality, the finding of various combinations of hypochromia, target cell formation, microcytosis and basophilic stippling should strongly suggest the possibility of thalassemia minor. The most common, but not absolutely constant, hematologic diagnostic feature of thalassemia minor is an increase in the hemoglobin A_2 fraction. The normal value for this fraction varies somewhat from one laboratory to another because of technologic differences but is usually from 1 to 2.5% of the total hemoglobin. Hemoglobin A_2 is absent in the blood of the newborn, is rarely increased in hematologic disorders other than thalassemia minor and has been reported to be elevated in only a few patients with thalassemia major. The reports of Rucknagel and Neel (46) of a lesser incidence of elevated hemoglobin A_2 fraction in the American Negro with thalassemia minor (as compared with persons of Mediterranean origin with this disorder) and the report of Ceppelini (5) of a minor hemoglobin fraction termed hemoglobin beta-2 (β_2) (which may be formed by an allele of the hemoglobin A_2 determinant) indicate that thalassemia minor encompasses diverse disorders.

The term "thalassemia intermedia" is used to describe patients

who have overt hemolytic anemia, erythrocyte morphologic alterations which are more severe but similar in nature to those seen in thalassemia minor, and a clinical course similar to, but more benign than, that observed in classic thalassemia major. This category is now known to include patients with thalassemia and one of the other hemoglobinopathies. Relatively high gene frequencies for hemoglobins S, C, D, G and H exist among many of the populations which have incidences of thalassemia. It is not surprising, therefore, that both the thalassemia gene and that of another hemoglobinopathy should occur in one person. The more frequent combinations of the double heterozygous abnormality are those of thalassemia–sickle cell disease, thalassemia–hemoglobin C disease, thalassemia–hemoglobin H disease and thalassemia–hemoglobin G disease. Pearson, Gerald and Diamond (42) have reported the occurrence of thalassemia intermedia due to the combination of Lepore trait and thalassemia trait.

Most of the clinical and physiologic descriptions of sickle cell anemia are pertinent to thalassemia–sickle cell disease. The hemolytic anemia may be less severe, the thrombotic events less frequent and the painful crises less common in thalassemia–sickle cell disease than in sickle cell anemia. Many observers have noted a rough correlation between the severity of the clinical disease and the proportion of sickle cell hemoglobin present in the erythrocytes, but important exceptions to this general impression have been noted. The major manifestations of the thalassemia disorders are listed and compared in the accompanying table. Clinically, thalassemia-hemoglobinopathy disorders other than thalassemia–sickle cell disease are remarkably similar to, but much less severe than, thalassemia major and must be differentiated on the basis of a thorough examination of the patient, parents, siblings and offspring of the patient and study of their hemoglobins by a variety of methods.

Thalassemia major is a grave illness, which is manifest early in childhood by severe hemolytic anemia, retarded physical development, hepatosplenomegaly and in some cases by a rather mongoloid appearance. There is an increase in the fetal hemoglobin fraction in all patients with this disorder. The proportion of fetal hemoglobin varies considerably from one patient to another in a range of 10–90%, and there is little correlation between the

CLINICAL FINDINGS OF ELEVEN OF THE MORE COMMONLY OCCURRING HEMOGLOBINOPATHIES

Disease	Alphabet Designation	Polypeptide Designation	Racial or Geographic Predominance	Severity of Anemia†	RBC Morphology	Hepato- and Splenomegaly†	Hemolytic Anemia†	Thrombosis†	Aplastic Crisis†	Ocular Manifestations†	Effect of Splenectomy
HOMOZYGOUS HEMOGLOBINOPATHIES											
Sickle cell anemia	SS	$\alpha_2^A \beta_2^S$	Negro	++++	Sickle cells, target cells	Hepatomegaly only	++++	++++	++++	+++	None
Thal. major	Th.-Th.	$\alpha_2^A \beta_2^F$	Medit., Negro, Asia	++++	Target cells, microcytosis, hypochromia	++++	+++	±	±	–	Occasional benefit
Hb C	C-C	$\alpha_2^A \beta_2^C$	Negro and Caucasian	++	Target cells, hypochromia	+++	++	+	......	......	Occasional benefit
Hb D	D-D	*	Negro, Indian	+	Target cells	......	+	......	......	......	None
Hb E	E-E	$\alpha_2^A \beta_2^E$	Southeast Asia, Thai	+	Target cells, microcytosis	±	......	......	......	......	None
DOUBLE HETEROZYGOUS HEMOGLOBINOPATHIES											
Hb S–C	S-C	$\alpha_2^A \beta_1^S \beta_1^C$	Negro	+++	Sickle cells, target cells, hypochromia	++	++	++	++	++++	None
Hb S–thal.	S-Th.	*	Negro and Medit.	+++		++	++	+	+	++	None
Hb S–D	S-D	*	Negro and Medit.	+++	Sickle cells, target cells	±	+++	++	++	++	None
Hb C–thal.	C-Th.	*	Negro and Medit.	±	Microcytosis, target cells	++	+	......	......	......	None
Hb E–thal.	E-Th.	*	Southeast Asia and Medit.	++	Hypochromia, microcytosis	+++	++	......	......	......	Occasional benefit
Hb H–thal.	H-Th.	*	Medit. and Southeast Asia	++	Target cells hypochromia, microcytosis	+++	++	......	......	......	Occasional benefit

*Either inadequate data for characterization or multiple possibilities have been postulated.
†The symbol ++++ refers to the disease in which the finding appears most commonly; other gradations are relative to this.

amount of fetal hemoglobin and the severity of disease. The remainder of the hemoglobin is type A except for a normal amount of hemoglobin A_2. The extent of the chemical similarity between the alkali-resistant fetal hemoglobin of the patient with thalassemia major and that present in the blood of the newborn is still unknown. Fetal hemoglobin from these two different sources are identical in many respects but have been reported to have different immunologic properties (36).

The severe hemolytic anemia which is present is accompanied by changes in the erythrocyte morphology of polychromasia, basophilic stippling and anisocytosis. There are also increased numbers of reticulocytes, and nucleated red blood cells appear in the peripheral blood. Of importance is the characteristic hypochromia and microcytosis of the red blood cells. The hypochromia of erythrocytes in the presence of normal or increased amounts of body iron has led to the suggestion that the anemia of thalassemia is due not only to a shortened life span of the red cell but also to a decreased rate of red cell synthesis. Measurements of the rates of red cell production and destruction in four patients with thalassemia major were made by Sturgeon and Finch (51). In these four patients, red cell destruction was accelerated in similar degree and the capacity to produce red cells and deliver them to the peripheral circulating red cell mass was variably reduced. The suboptimal erythropoietic response to accelerated blood destruction is an important physiologic deficit in the patient with thalassemia major. To investigate the nature of the defect in erythrocyte production in thalassemia, Bannerman, Grinstein and Moore (3) have made comparisons of the rates of incorporation of tagged glycine and iron into heme, globin and hemoglobin by reticulocyte-enriched samples of blood from normal persons and patients with thalassemia major under in vitro conditions. The results of these studies indicate that there is a defect in either the ability to synthesize porphyrin or the ability to associate iron with protoporphyrin in the formation of heme by the erythrocytes of patients with thalassemia. The exact manner in which the abnormalities of hemoglobins, of red cell morphology and of altered red cell production and destruction combine to form the clinical picture of thalassemia is unknown.

CONSIDERATION OF POPULATION GENETICS

The recognition of abnormal hemoglobins as products of mutant genes (as contrasted with normal hemoglobins, which are the products of the more common types of genes), the ease of identification of abnormal hemoglobins by electrophoresis and the high incidence of hemoglobinopathy among certain populations have provided opportunity for the study of certain basic problems of human genetics. A detailed and informative discussion of the progress and results of these studies has recently been published by Rucknagel and Neel (46).

In any reasonably stable population with a high incidence of sickle cell trait, the problem arises as to the mechanisms which maintain the high incidence. While there is little evidence of increased morbidity or mortality in sickle cell trait, the primitive conditions surrounding some of these populations make likely the death of all persons with sickle cell anemia before they could produce offspring. Thus, there must exist a constant depletion of sickle cell genes (via death of persons homozygous for sickle cell hemoglobin), which, if unbalanced, would reduce the sickle cell gene frequency of that population. Many workers (46, 52) have contributed to the studies, which allow tentative impressions that the loss of sickle cell determinants is balanced by certain selective advantages afforded the person with sickle cell trait over the person with the normal hemoglobin. In this very complex problem, which has numerous facets and ramifications, there is now substantial evidence that the person with sickle cell trait, in contrast with the person with normal hemoglobin, has decreased susceptibility to experimentally induced P. falciparum infections, has a lesser degree of parasitemia when the spontaneous infection is present and has a lesser mortality from this type of malaria. The physiologic or biochemical reasons for the resistance of the person with sickle cell trait to P. falciparum but not to other types of malaria are unknown.

TREATMENT

Patients with sickle cell trait, thalassemia minor or other less common single heterozygous hemoglobinopathies which are ac-

companied by few or no symptoms need no active form of therapy. Indeed, one very important and practical advantage which results from the accurate diagnosis of trait states is the prevention of unwarranted, misdirected diagnostic procedures and unwarranted iron, vitamin or transfusion therapy.

A comparison of the therapy of sickle cell anemia as discussed by Margolies (37) a decade ago with the current management reveals a striking similarity and serves to emphasize the need for more intimate knowledge of the intermediary steps of the pathology which result in multiple thromboses and hemolytic anemia. Specific management of the patient with sickle cell anemia cannot be adequately outlined because of the wide variety of clinical diseases which these patients exhibit. Some general principles which apply to a majority of patients may, however, be offered. During asymptomatic periods, the patient does not require therapy. The patient who is without the symptoms of anemia or other acute disease should not be given transfusions, despite laboratory evidence of moderate anemia. Indiscriminate blood therapy serves only to increase the incidence of complications when transfusions become necessary.

Prompt recognition, specific identification and appropriate treatment of infections are mandatory to optimal care, for, despite a lack of knowledge concerning the relation of infection to the onset of painful crises or vascular thrombosis, these events are not uncommonly related.

Adequate exchange of respiratory gases is of prime importance to all persons; but it is of critical importance to the patient with sickle cell anemia, since desaturation of the hemoglobin provides a basis for increased intravascular sickling of erythrocytes. The occurrence of respiratory tract disease, whether infectious, thrombotic or ischemic, demands immediate attention. The use of oxygen, bronchodilators, tracheobronchial suction, tracheostomy and intercostal nerve blocks to maintain adequate respiratory gas exchange are extremely important facets of optimal care. It is during the critical periods of pulmonary, cardiovascular, abdominal or ocular symptoms that transfusion and oxygen therapy are of greatest value. Despite the known suppression of erythropoiesis induced by prolonged oxygen administration and by transfusions, the intelligent use of these agents constitutes the major effective

therapy available for the acutely ill patient wih sickle cell anemia.

Periods of accelerated rates of erythrocyte destruction are rarely seen in sickle cell anemia, but sudden decreases in the red cell mass are not uncommon. A decrease in erythrocyte concentration is usually associated with other complicating illness or idiopathic aplasia of the bone marrow. Episodes of bone marrow aplasia are usually transient and require blood transfusions. Hematinics and corticosteroids are usually not of therapeutic value. Aplastic crises should be differentiated from the megaloblastic anemia which occurs occasionally in the more severe chronic hemolytic anemias, since the latter respond satisfactorily to the oral or parenteral administration of folic acid. Other than in this particular circumstance, vitamin and mineral dietary supplements have no specific role in the management of sickle cell anemia. The oral administration of iron is unwise, since it may aggravate pre-existent hemosiderosis. The list of medications which have been thought to have benefit, but which on prolonged trial were found to be ineffective, has increased and now includes anticoagulants, adrenal cortical steroids and acetazolamide (15). Greenberg and Kass (11) have noted salutory effects in some patients with sickle cell crises after intravenous administration of alkaline salts.

Splenectomy is of value in selected patients. Decision to perform splenectomy is probably warranted only when the spleen is appreciably enlarged and there is evidence of "hypersplenism." An overactive spleen may be indicated by leukopenia, pancytopenia, premature destruction of transfused normal compatible erythrocytes and the splenic sequestration of such cells. It is apparent that the advent of any combination of such findings necessitates a search for the causes of either isoimmune or autoimmune hemolytic anemia.

The management of the problems of genitourinary tract hemorrhage, priapism, aseptic necrosis of the hip, hemorrhages into the ocular vitreous and the advent of pregnancy require the combined efforts of physicians with special interests.

The management of thalassemia intermedia consists primarily of the use of blood transfusions. Transfusions are seldom necessary and should be given only when the patient's symptoms indicate a need for an increased oxygen-carrying capacity. The important, more specific aspects of therapy of thalassemia major are

blood administration and splenectomy. These have been discussed by Smith, Erlandson, Stern and Schulman in a recent publication (49). These investigators recommend the use of transfusions to maintain hemoglobin concentrations near 6.5–8.0 Gm./100 ml. This range provides relative symptomatic relief to a majority of patients and requires fewer transfusions than would be necessary to achieve higher and perhaps unnecessary hemoglobin concentrations. Not uncommonly, patients with thalassemia major develop an acquired extracorpuscular hemolytic anemia in addition to their primary disease. The pathogenesis of the extracorpuscular hemolytic anemia is often unknown; but it is made manifest by the increased needs for transfusions, the intractability of symptoms of anemia and the progressive enlargement of the spleen. The extracorpuscular hemolytic anemia may be documented by a demonstration of the shortened life span of transfused normal erythrocytes in the patient. Patients with thalassemia major who develop acquired extracorpuscular hemolytic anemia usually achieve benefit by splenectomy. Because a few children who have undergone splenectomy have increased susceptibility to infections, the use of prophylactic antibiotics after splenectomy is recommended.

Vitamin and mineral supplements, corticosteroids and anticoagulants have no specific benefit in thalassemia major. Iron therapy is specifically contraindicated because most patients with thalassemia major have marked hemosiderosis.

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